Environmental Temperature and Relative Humidity, two Key Factors in Maize Technology Affecting Ochratoxin a Production and Growth of Ochratoxigenic Species

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Abstract—Ochratoxin A (OTA) is a secondary fungal metabolite produced naturally by filamentous fungi of the Aspergillus and Penicillium genera. The aims of this study were to identify the most relevant OTA producing species in maize in Spain by PCR and to study the effect of environmental conditions (a_w, temperature) on the development of these fungi and on OTA production in maize.

Eighteen and 20 corn samples harvested in 2014 and 2015, respectively, from conventional farms and 31 and 34 corn samples harvested in 2014 and 2015, respectively, from organic farms located in various Spanish areas were tested for presence of ochratoxin-producing fungi. The most important OTA-producing Aspergillus spp. were isolated and identified by species-specific PCR protocols. The growth rate of the main ochratoxigenic species (A. steynii and A. ochraceus) under different environmental conditions was registered and OTA level in cultures was determined by solvent extraction, immunoaffinity column clean-up and LC-fluorescence detection. The results revealed that A. steynii and A. ochraceus are good indicators of OTA risk in maize. Temperature, a_w, and isolate influenced OTA production. The highest OTA levels were found in maize cultures of A. steynii at 0.98 a_w and 30 ºC.

Index Terms—Ochratoxin A, ochratoxigenic Aspergillus, maize, environmental conditions, food safety, risk assessment

I. INTRODUCTION

In spite of many years of research and the introduction of good agricultural practices in the food production, and good manufacturing practices in the storage and distribution chain [1], fungi and mycotoxins continue to be a problem in food and feed [2].

It is estimated that 25% of the world’s food production, including many basic foods, is affected by mycotoxin-producing fungi. Reports on mycotoxin occurrence on different cereals and by-products show that maize is the first most contaminated crop worldwide [3], [4].

Ochratoxin A (OTA) is produced by Aspergillus spp. sections Circumdati and Nigri in warm and tropical countries and in temperate climates it is produced by Penicillium verrucosum [4], [5]. The most important ochratoxigenic Aspergillus spp. are A. ochraceus, A. westerdijkiae and A. steynii, which belong to Aspergillus section Circumdati [6]. The second OTA-producing Aspergillus group is classified under Aspergillus section Nigri [7]. Aspergillus of sections Circumdati and Nigri have been isolated from cereals [8]-[11]. The main sources of daily OTA intake are cereals and cereal products, followed by wine, grape juice and coffee [8]-[13].

OTA is one of the most important mycotoxins in food and feed. IARC has classified this mycotoxin as a probable human carcinogen, in group 2B [14]. Previous studies have shown that a number of diseases are associated with OTA exposure in both animals and humans, predominantly affecting the kidney. It is also associated with other toxic effects such as neurotoxicity, reproductive toxicity, myelotoxicity, immunotoxicity, and teratogenicity [15]-[17].

In Spain, Mateo et al. [8] observed a high occurrence of Aspergillus spp. of sections Circumdati and Nigri in barley crops, which represent up to 40% of the Spanish cereal production. The presence of these fungi in pre- and postharvest barley grains indicates their adaptation to warmer weather. Magan et al. [18] suggested that climate change toward hot temperatures and drought could increase the risk of pathogen migration.
The health-beneficial properties of maize have led to an increase in the consumption of maize-based food products in recent years. Due to the dietary importance of maize and its by-products, it is worth to know and to control the ochratoxigenic mycobiota of this cereal, its distribution in the different agro-climatic regions worldwide and the environmental conditions that affect mycotoxin production in the cereal. The presence of a particular ochratoxigenic mould does not always indicate that OTA occurs. The contrary situation is also true: since OTA is stable, it might be detected long after the producing fungi have died out or have been outgrown by other species [3], [19], [20].

The interest in the area of food safety is increasing and traditional methods for detection and identification of possible ochratoxigenic fungi in food is laborious and often difficult even for expert taxonomists. New methods based on the Polymerase Chain Reaction (PCR) are rapid, specific, and highly sensitive, being able to detect target DNA molecules in complex mixtures even when the fungi are no longer viable. Several PCR assays have been developed to detect OTA-producing Aspergillus spp. using as target constitutive genes or toxin biosynthetic genes [21], [22]. However, the sensitivity of the assay can be considerably improved when multi-copy sequences, such as ribosomal DNA regions, are used. Both ITS1 and ITS2 (Internal Transcribed Spacers or the rDNA) regions are highly variable in Aspergillus genus allowing discrimination among closely related species [23].

The aims of this work were: i) To determine the most important Aspergillus spp. that are potential producers of OTA in contaminated Spanish maize samples by species specific PCR protocols based on multi-copy sequences (ITS); and ii) To test the impact and effect of environmental conditions associated to storage on both the development of these ochratoxigenic moulds and OTA production in maize.

II. MATERIALS AND METHODS

A. Mycological Study of Positive Samples

During 2014 and 2015 a total of 103 samples of corn were collected from 16 Spanish farms: 38 samples of corn were collected from 7 farms using conventional cultivation methods, and 65 samples were collected from 9 organic farms. These maize samples were collected in farms that showed a strong desire to cooperate in the present study and facilitated regular assistance with sampling. On the other hand, maize grains from organic crops were more susceptible to fungal attack and exhibited more variability on particular agricultural practices. Thus, a larger number of samples from organic crops was examined to generate adequate and sufficient database.

No samples were mouldy in appearance. The farms were located in wetland Spanish areas (Galicia, Asturias, Santander, Basque Country and “Albufera” of Valencia), where maize grows without artificial irrigation, and in dry areas or irrigated areas (Aragón, Castilla, Extremadura and Andalucia). Maize samples were taken 1 to 3 weeks after harvest. Sampling was performed following the protocol described in Commission Regulation (EC) No 401/2006. Representative subsamples of 1000g each were collected from each lot of cereal and homogenized to obtain a final sample of 10kg. The samples were sent to the laboratory and final subsamples of 500g were prepared and immediately processed for mycological analysis.

B. Isolation and Identification of Ochratoxigenic Fungi and Determination of Ochratoxin Producing Potential of the Isolates

To reduce surface contamination 100 g of each maize sample were surface-sanitized with 0.5L of a 2% solution of sodium hypochlorite for 1 min. Afterwards, the grains were washed twice with 0.25L of sterilized pure water for 1 min. Fifty seeds per sample were placed on 9 cm diameter Petri dishes (5 seeds/dish) filled with Dichloran Chloramphenicol Peptone Agar (DCPA) [16] modified using 15% less quantity of chloramphenicol and incubated at 25°C for 7 days. Based on their phenotypic characteristics, colonies identified at the section level as Aspergillus spp. section Circumdati and Aspergillus spp. section Nigri were selected and single spore cultures were prepared in Czapek Agar for identification. After 7 days, identification was carried out by species-specific PCR protocols.

OTA-producing Aspergillus species, A. westerdijkiae, A. ochraceus, and A. steynii following the methodology reported by Gil-Serna et al. [6]. PCR assays specific for these OTA-producing species, on the basis of the multicopy ITS regions of the rDNA were used. All genomic DNAs used in this work were tested for suitability for PCR amplification using primers ITS1 and ITS4, which amplify the ITS1-5.8S-ITS2 region. The specific PCR amplification protocol to detect A. westerdijkiae, using specific primers set WESTF/WESTR was as follows: 5 min at 95°C, 22 cycles of 30s at 95°C (denaturation), 30 s at 63°C (annealing) and 40 s at 72°C (extension) and, finally, 5 min at 72°C. OCRAF/OCRAR primers were used to detect A. ochraceus according to the following PCR program: 5 min at 95°C, 24 cycles of 30 s at 95°C, 30 s at 62°C and 40 s at 72°C and, finally, 3 min at 72°C. For A. steynii, STEYF/STEYR primers were used. In this case, the amplification protocol was as follows: 5 min at 95°C, 21 cycles of 30 s at 95°C (denaturation) and 45 s at 72°C (annealing and extension) and, finally, 5 min at 72°C. WESTF/WESTR and OCRAF/OCRAR primers amplified a single fragment of about 430 bp only when genomic DNA of A. westerdijkiae or A. ochraceus was present, respectively. STEYF/STEYR primers amplified a single fragment of 315 bp only when genomic DNA of A. steynii isolates was present.

A. carbonarius, A. niger, and A. tubingensis were identified following the methodology reported by Medina et al. [7] and González-Salgado et al. [24]. A. carbonarius can be microscopically distinguished by conidial size and ornamentation and by PCR protocols using the CAR1/CAR2 primer pairs [24]. However, all the taxa in the A. niger aggregate are morphologically indistinguishable. Identification of isolates of A. niger...
aggregate (A. niger and A. tubingensis) was based on PCR amplification of 5.8S rRNA genes and the two intergenic spacers, ITS1 and ITS2, followed by the subsequent digestion of these PCR products with restriction endonuclease RsaI [7]. Digestion was carried out overnight at 37°C with RsaI (Boehringer Mannheim). PCR products and restriction fragments were separated by electrophoresis in 1% and 2% agarose gels, respectively, with 0.5 x Tris-borate-EDTA buffer. After electrophoresis, gels were stained with ethidium bromide (0.5 μg/ml) and the DNA bands were visualized. The PCR-amplified 5.8S rRNA gene of A. niger is digested into two fragments of 519 and 76 bp. The 76-bp fragment was too small to remain on the gel. The PCR-amplified 5.8S rRNA gene of A. tubingensis was not digested by RsaI. In this latter case a band of 600 bp is observed.

C. Effect of Environmental Conditions on Growth and OTA production by A. steynii and A. ochraceus in Maize

Three isolated of A. steynii (AsM02, AsM11, and AsM09) and three isolates of A. ochraceus (AoM07, AoM05, and AoB13) were selected to study the effect of environmental conditions on fungal growth and OTA production in maize grains. These strains are held in the Mycology and Mycotoxins Group Culture Collection (Valencia University, Spain).

D. Growth Evaluation

Maize kernels (15g), previously analyzed to ensure they had undetectable levels of OTA were placed in Erlenmeyer flasks and autoclaved for 20 min at 121°C. Then, water activity (a_w) was adjusted to 0.96 and 0.98 by addition of sterile pure water. Flasks containing maize grains were refrigerated at 4°C for 48 h with periodic shaking to allow for water adsorption and equilibration. At the end of this period, a_w-values were checked. Fifteen g of the hydrated maize grains were placed in sterile 9-cm Petri dishes to form a layer. All treatments were centrally inoculated with 3-mm diameter agar plugs taken from the margin of 5-7-day-old growing colonies of A. steynii and A. ochraceus. Inoculated Petri dishes of the same a_w were enclosed in sealed plastic containers together with beakers of a glycerol-water solution matching the same a_w as the treatments to maintain a constant equilibrium relative humidity inside the boxes. The experiments were carried out at two temperatures, 20°C and 30°C, for 14 days. The experiments were carried out in triplicate and repeated once. Assessment of growth was made every day during the incubation period by measurement of two diameters of the growing colonies at right angles to each other until the colony reached the edge of the plate. The radii of the colonies were plotted against time, and linear regression was applied. The growth rate (mm/day) was estimated by the slope of the line. After 14 incubation days, three replicates per treatment were dried at 50°C for 24h, milled, and stored at -20°C before OTA determination.

E. OTA Determination

To find OTA level in maize seed cultures, all the kernels distributed as a homogenous layer on each Petri dish were used regardless of the colony diameter reached after 14 incubation days. Grains were milled, homogenised and analyzed for OTA by an optimized and validated method involving accelerated solvent extraction, clean-up by immunoaffinity column, liquid chromatographic separation and fluorescence detection, as described by Mateo et al. [8].

A Dionex Accelerated Solvent Extraction (ASE 150) system (Dionex Co., Sunnyville, CA) was used for extraction of OTA. Ten grams of ground sample was weighed and mixed with 3 g of diatomaceous earth drying matrix (Dionex). The sample was packed into the 22-ml extraction cell, and the void volume on the top was filled with glass beads. A mixture of acetoniitrile–water (60:40, v/v) was used for OTA extraction. Temperature was 100°C and pressure was 10.3 MPa. Fifty mL of extract (2x25 mL) was collected and let stand overnight at 4°C to allow for protein precipitation. After filtering through paper filter the clear extract was concentrated in a rotary evaporator to near dryness and re-dissolved with acetoniitrile-water (60:40, v/v) to a final volumen of 10 mL. Then, 2.5 mL was transferred to a 50-mL measuring flask and diluted with Phosphate-Buffered Saline (PBS) solution (2.5 g sample/50 mL). Clean-up was carried out using OchraTest immunoaffinity columns (Vicam, Watertown, MA). The column was first conditioned with PBS (20 mL). Then, 20 mL of diluted sample extract (1.0 g sample) was placed on the column and made to flow at a rate of no more than 3 mL/min. The column was washed with 10 mL pure water (Milli-Q) and then dried by passing air through the column (approximately 3 mL) to remove any remaining water prior to quantitative elution. OTA was eluted with methanol (4 mL). The solvent was carefully evaporated under a slight stream of N_2 at 40°C. The residue (1.0 g sample) was dissolved in 0.5 mL methanol–2% aqueous acetic acid (1:2), filtered through 0.22 μm filter into a vial and used for liquid chromatographic analysis.

Chromatographic conditions were as follows: the LC system consisted of a Waters 600 pump, a Waters 717 automatic injector and a Waters 474 scanning fluorescence detector (Waters Co., Milford, MA, USA). Separation was performed on a Phenomenex Gemini C18 column (150 x 4.6 mm, 5 μm particle size) (Phenomenex Inc., Torrance, CA, USA). The LC system was controlled and signals were processed by Millennium 32® software version 3.01.05 (Waters). Column temperature was 35 °C. Separation was carried out at a flow rate of 1 mL/min using water-acetonitrile-acetic acid (41:57:2, v/v/v) as a mobile phase. The mobile phase was filtered through a 0.45-μm filter and degassed before use. Retention time for OTA was 5.1 min. Excitation and emission wavelengths were 330 and 460 nm, respectively.

F. Statistical Analysis

The data were treated by multifactor ANOVA and Duncan’s test of multiple comparisons using Statgraphics Centurion XV.2.11 software (Statpoint Inc., VA, USA). A 95% confidence level was used to assess influence of individual and interacting treatments. For calculation purposes, detectable OTA levels below the limits of...
quantification (LOQ) were estimated as 50% of those limits and undetectable levels were assumed to be zero. Logistic regression of OTA presence (0 = below LOD, 1 = above LOD) against presence of target DNA of ochratoxigenic Aspergillus spp. was carried out by categorizing the DNA variable as 0 = not detected or 1 = detected.

III. RESULTS AND DISCUSSION

The Aspergillus spp. isolated from maize kernels and their ability to produce OTA are summarized in Tables I and II. Kernels were also infected by other fungal species belonging to the genera Fusarium, Alternaria, Cladosporium, Epicoccum, Mucor and Rhizopus.

Identical fungal genera were isolated from corn grown in both farming systems and geographic regions.

The percentage of infected samples with fungi was 100% in all cases. The predominant fungus in maize samples was Alternaria spp., followed by Fusarium spp., Aspergillus spp. and Penicillium spp., which agrees with results reported by other authors [25]-[27].

The occurrence of Aspergillus in seeds was higher in dry than in wet regions. Environmental conditions in dry regions offer obvious advantages to more xerotolerant fungi Aspergillus spp. over other less competitive species. Desertification and fluctuations in wet-dry cycles, associated to climate change will have a strong impact on the life cycle of all microorganisms including toxigenic moulds and their patterns of mycotoxin production in crops. In terms of total maize kernels infected with Aspergillus spp. ANOVA showed that there were significant differences (p < 0.05) with regard to the geographic origin of the samples and production system (organic or conventional) (Table I).

Only three ochratoxigenic species were identified by PCR protocols in the analyzed maize samples: A. niger, A. steynii and A. ochraceus. OTA producing potential of the isolates from these species is shown in Table II. OTA was produced mainly by A. steynii isolates following by A. ochraceus and A. niger isolates. The percentage of OTA producing isolates from conventional and organic maize farms was for A. steynii, 87.5 and 100%; for A. ochraceus 33.3 and 36.4%, and for A. niger 30.0 and 33.3 %, respectively. Moreover, very low OTA concentrations were found in A. niger cultures and frequently such levels varied between the LOD and LOQ (0.05 and 0.17 ng/g, respectively). These results lead to the conclusion that A. steynii and A. ochraceus are the main species involved in the production of OTA in the studied maize samples.

The effect of environmental conditions on the growth rate and OTA production of three selected isolates of A. steynii and A. ochraceus in maize can be observed in Figs. 1 and 2. ANOVA revealed that aw and temperature and their interaction had a significant effect on growth responses (p < 0.05). In general, growth was faster at 30 ºC than 20 ºC, regardless of aw, and at 0.98 than 0.96 aw, regardless of temperature. Under the same conditions, no significant differences were found among the isolates of both species regarding growth kinetics (Fig. 1).

Fig. 2 shows the effect of temperature and aw on OTA concentration (µg/kg) in all maize cultures of the six isolates under all the assayed conditions. ANOVA showed that aw, temperature and isolate and their two and three way interactions had a significant effect on OTA production (p < 0.05). Usually, OTA levels were much higher at 30 ºC than 20 ºC, regardless of aw, and at 0.98 than 0.96 aw, regardless of temperature, and higher by A. steynii than by A. ochraceus regardless of aw and temperature. The highest OTA levels were found in maize cultures of A. steynii at 0.98 aw and at 30 ºC.

No previous report has examined the impact that environmental conditions have on growth of A. steynii and A. ochraceus isolated from Spanish maize and on OTA production in maize kernels. As far as we know, only a study on wheat has been published [28] using ochratoxigenic isolates obtained from other hosts (barley and grapes). In that paper [28], very high OTA levels were found in wheat cultures of A. steynii incubated at 25/35 ºC cycle, which surpass 1 mg/kg. The assays were performed by inoculation of seeds on the dish center with 5 µL of a spore suspension of fungi of 10^6 spores/mL. When cereal grains are used as culture media, this technique generally produces a lag phase (time spent for the colony to reach 2-3 mm diameter) of several days.

### Table I. Aspergillus spp. occurrence in conventional and organic maize samples harvested in 2014 and 2015 in different Spanish regions

<table>
<thead>
<tr>
<th>Spanish region</th>
<th>Production system</th>
<th>No. of samples 2014+2015</th>
<th>Total No. of kernels</th>
<th>% Infected kernels mean (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet regions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conventional</td>
<td>10+11</td>
<td>1050</td>
<td>5 (1-14)</td>
<td></td>
</tr>
<tr>
<td>Organic</td>
<td>19+21</td>
<td>2000</td>
<td>10 (5-13)</td>
<td></td>
</tr>
<tr>
<td>Dry regions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conventional</td>
<td>8+9</td>
<td>850</td>
<td>13 (1-22)</td>
<td></td>
</tr>
<tr>
<td>Organic</td>
<td>12+13</td>
<td>1250</td>
<td>18 (5-28)</td>
<td></td>
</tr>
</tbody>
</table>

### Table II. Ochratoxin A producing potential of fungi isolated from conventional and organic maize samples harvested in different Spanish regions

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Toxin</th>
<th>No. of assayed isolates (% positive)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Conventional maize</td>
</tr>
<tr>
<td>A. niger</td>
<td>OTA</td>
<td>10 (30.0)</td>
</tr>
<tr>
<td>A. steynii</td>
<td>OTA</td>
<td>8 (87.5)</td>
</tr>
<tr>
<td>A. ochraceus</td>
<td>OTA</td>
<td>6 (33.3)</td>
</tr>
</tbody>
</table>
However, those authors found average growth rates, for example, for A. carbonarius, higher than 7 mm/day during the first incubation week, which do not agree with results of the present study.

More studies are needed to clarify the effects of temperature and relative humidity on the growth of ochratoxigenic fungi and OTA production in cereals, as these commodities represent the main dietary source of OTA. Ecological studies on the effect of environmental conditions on growth and T-2 and HT-2 production by Fusarium langsethiae isolated from oats in England, Finland, Norway and Sweden have been carried out [29]-[31]. It was suggested that there is little difference between F. langsethiae strains from different European countries in terms of ability to grow under interacting extrinsic environmental factors but there are differences related to $a_w$ and temperature. Comparative studies on growth profiles and mycotoxin production by F. langsethiae related to $a_w$ and temperature in oat-based media and oats show that the growth rate and production of T-2 and HT-2 are significantly lower in the cereal than in culture media made with cereal extracts under all the assayed conditions [31], [32].

In summary, very few studies have examined the impact that interacting environmental conditions can have on growth of fungal pathogen and mycotoxin production in cereal grains. The present study has shown, for the first time, data on the influence of interacting ecological factors on growth and OTA production by A. steynii and A. ochraceus in maize. Both environmental factors, $a_w$ and temperature, appear to be very important in determining accumulation levels of OTA in this cereal. Consequently, global warming and increasing rain will devise a scenario that will favour OTA production by ochratoxigenic species that contaminate maize, particularly, A. steynii, a high OTA producer.

Although the complete elimination of mycotoxins in maize is not achievable at this time, the results of the present study show that implementation of Good Agricultural Practices (GAP) and Good Manufacturing Practices (GMP) to minimize fungal growth and mycotoxin occurrence in the cereal is needed. A Code of practice for the prevention and reduction of mycotoxin contamination in cereals [33], was adopted in 2003, amended in 2014 and revised in 2016, in the frame of climate change. It includes recommendations to be adopted in different stages of the crop, such as planting and crop rotation, tillage and preparation for seeding, pre-harvest, harvest, drying and cleaning before storage, storage after drying and cleaning, transport from storage and processing and cleaning after storage.

ACKNOWLEDGEMENT

The authors acknowledge financial support from FEDER and MINECO (Spanish Government) (Project AGL2014-53928-C2-1-R). Eva M. Mateo is grateful to ‘Generalitat Valenciana’ for financial support (contract APOSTD/2016/102). Jose V. Gómez is grateful to FEDER and MINECO for a Ph.D. contract.

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Dr. Eva M. Mateo is Graduate and M.C. in Pharmacy and Ph.D. in Microbiology (2015) from University of Valencia (Spain). Her international doctoral thesis obtained the Extraordinary Doctorate Award. She has been awarded by different Spanish Ministries with numerous fellowships: Collaboration Student, Research Staff Training (FP1), International Mobility for Researchers and Post-Doctoral Contracts. She has acquired a solid multidisciplinary training in the fields of food microbiology, quality and safety, analytical chemistry and management skills and clinical microbiology. Her research has been mainly focused on: a) conventional and molecular characterization of toxigenic fungi, b) prediction of the effect of the climate change on fungal growth and mycotoxin production in relevant products to human diet, c) antifungal and antibacterial susceptibility testing, d) design of high-resolution chromatographic techniques for the determination of mycotoxins, e) incidence of the most relevant human viruses and f) training for personnel responsible for the design and management of procedures with animals (group C). Dr. Mateo has developed these researches in the ‘Mycology and Mycotoxin’ Group (Department of Microbiology and Ecology, University of Valencia, Spain), ‘Applied Mycology Group’ (Cranfield Health, Cranfield University, UK), ‘Fungi and Yeast in Food Group’ (Department of Genetics, Complutense University of Madrid, UCM, Spain) and the Valencian Health Research Institute (INCLIVA), internationally recognized research groups and institutions. Dr. Mateo is the author of 20+ scientific articles (ranked Q1, according to SCI JCR), 20+ book chapters (international edition), numerous contributions presented at national (10+) and international conferences.
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Dr. David Romera graduated in both Chemistry in 1997 and Food Science and Technology in 2007 from University of Valencia. Ms.C. in Optical Technologies Applied to Industry in 2008. He earned his Ph.D. in Chemistry in 2016 from University of Valencia. He worked as a Specialist Technician in Food Safety at the ‘Generalitat Valenciana’ (Valencian Autonomous Government, Spain), Chemist at the Agro-Food Laboratory (EU Reference Laboratory of Residues in Food and Vegetables, DG SANCÉ, European Commission-Generalitat Valenciana’). Currently, he is Research Chemist at the ‘Conselleria de Sanitat’ (‘Generalitat Valenciana’). He is author of research papers presented at national and international Conferences.

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Prof. Jose V. Gimeno-Adelantado graduated in Chemistry from University of Valencia. He joined the Department of Analytical Chemistry and completed his Ph.D. in Chemistry in 1981 from University of Valencia. He was Lecturer from 1973 to 1989, Associate Professor in 1989 and Full Professor since 2009 at the Department of Analytical Chemistry (University of Valencia). His research since the 80s has focused on the development of analytical methods for samples of mineral and organic origin using electron microscopy, spectroscopic and chromatographic techniques. In the last two decades he has dedicated his research to the analytical study of mycotoxins in food, as well as the study of materials of artistic and archaeological interest, also including electrochemical techniques. This research is carried out in collaboration with the group ‘Mycology and Mycotoxins’ of the Department of Microbiology and Ecology (University of Valencia) and the Institute of Heritage Restoration from Polytechnic University of Valencia. He has been senior researcher in some national projects. He has published 150 articles in high-ranking journals, numerous book chapters and 300+ contributions to conferences and symposia.

Prof. Rufino Mateo-Castro graduated in both Chemistry and Pharmacy from University of Valencia. He joined the Department of Analytical Chemistry and completed his Ph.D. in Chemistry in 1989 from University of Valencia. He was head of the departments of instrumental analysis at both the Laboratory of Animal Health and the Laboratory of Pesticide Residues in Vegetal Products (Ministry of Agriculture, Fisheries and Food, Spanish Government) at Valencia from 1976 to 2012. Since 1990 he shared his activities as officer in those centers with docent duties as part-time professor at the Department of Analytical Chemistry. Since 2012 he is Associate Professor at the Department of Analytical Chemistry (University of Valencia). He has been research collaborator in many competitive Projects sharing activities between the group ‘Mycology and Mycotoxins’ headed by Prof. M. Jiménez and the artwork analytical team headed by Prof. J.V. Gimeno-Adelantado and Prof. A. Doménech-Carbó (University of Valencia). He was member of the COST Action 835 (European Commission, 1999-2003). He has published more than 80 articles in JCR journals and 14 book chapters. He has also presented 130+ research papers at national and international symposia and conferences. At present, his main research interest includes mycotoxin analytical methods and biology of toxigenic fungi infecting food commodities.

Prof. Misericordia Jiménez graduated in Biology and Ms.C. in Microbiology from University of Valencia (Spain), she obtained her Ph.D. degree in Microbiology in 1987 from University of Valencia. The same year she created the ‘Mycology and Mycotoxins’ research group at the Department of Microbiology and Ecology (University of Valencia), where she is head of this group. This group is internationally recognized for research on ecophysiology and characterization (morphological, physiological and molecular) of toxigenic fungi in crops (cereals, citrus fruits, nuts, grapes…) and derivatives, design and optimization of analytical methods (GC, LC, GC-MS/MS, UHPLC-MS/MS, UHPLC-QTOF-MS) for determination of mycotoxins in food and feed, modelling of toxigenic fungi growth and toxin production (with especial attention to new scenarios associated to climate change), effects of food processing and agromonic factors on toxigenic fungi and mycotoxins, discovery of new strategies and bioactive compounds exhibiting antifungal activity. … She has lead numerous research projects about these topics and was member of the Management Committee of COST action-835 (European Commission, 1999-2003). Her group has published 250+ research papers (ICR), numerous book chapters and has been considered a group of excellence by the ‘Generalitat Valenciana’ (Spain). Dr. Jiménez is full professor at Valencia University in different degrees: Biotechnology, Biochemistry and Biomedicine, Biology and Environmental Sciences and has supervised numerous Ph.D. Theses and 150+ Training Projects (graduated student) in companies, hospitals and research institutes.